DNA AMPLIFICATION OF DNA USING GLOBALFILER

A. SCOPE

The GlobalFiler kit is a multiplex short tandem repeat (STR) system for use in DNA typing. This kit has a 6-dye configuration and amplifies 21 autosomal STR loci, Amelogenin, 1 Y-STR locus (DYS391), and 1 Y insertion/deletion (Y-indel) locus. The 21 autosomal STR loci are D3S1358, vWA, D16S539, CSF1PO and TPOX (labeled with 6-FAM); D8S1179, D21S11 and D18S51 (labeled with VIC); D2S441, D19S433, TH01 and FGA (labeled with NED); D22S1045, D5S818, D13S317, D8S820 and SE33 (labeled with TAZ); and D10S1248, D1S1656, D12S391 and D2S1338 (labeled with SID). The Amelogenin, DYS391 and the Y-indel markers are labeled with the VIC fluorescent dye. Ten of these STR loci are classified as mini-STRs because they are less than 220 bp and as such can perform well with degraded samples. All 24 loci are amplified simultaneously in a single well and can be analyzed in a single injection on an AB 3130 or 3500xL capillary electrophoresis instrument.

B. QUALITY CONTROL

B.1 Positive amplification/allelic control (e.g. DNA Control 007, 2800M etc.)

This sample ensures that the amplification and typing process is working properly. It is required to run a positive amplification control with each GlobalFiler amplification.

B.2 Negative controls:

Reagent Control: This is a tube containing no sample that is carried through the DNA typing process, involving all the reagents used for extraction, quantitation, and amplification. The purpose of this sample is to detect contamination that might occur from the reagents, the environment, or between the evidence samples being processed. At least two reagent controls will be extracted per extraction set, except during the extraction of reference samples where one reagent control may be extracted. All reagent controls will be quantitated, with the reagent control demonstrating the greatest signal being amplified and typed. A reagent control that is amplified and typed shall be amplified utilizing the same primers, instrument model, and concentration conditions as required by the evidence sample with the least amount of DNA; amplified with each amplification kit utilized; and typed using the same instrument model and injection conditions (i.e. injection times and voltage) as the associated evidentiary sample containing the least amount of DNA.

If an evidence sample is re-amplified with the same amplification test kit or system and the template volume is not increased over that of the original reagent control, then re-amplification of the associated reagent blank is not necessary.

Negative amplification control: This control contains only the reagents used to prepare the PCR amplification mixture for each batch of samples, including sample buffer (TE^{-4}) and/or water. The purpose of this sample is to detect contamination that might occur from the PCR reagents, the PCR setup environment or between the PCR reactions being prepared. It is required to run a negative amplification control with each amplification.

B.3 Water control:

Where appropriate, a deionized water control may be collected / submitted and run through the typing process to check for possible contribution to positive results from water. It is not a requirement that an analyst process the water control. If a water control is processed and the results of Plexor HY indicate "N/A", the sample contains no amplifiable DNA and the water control does not need to be carried through amplification. If the Plexor HY indicates a quantity, then the sample should be amplified and typed.

B.4 See DOC ID 1835 to determine reagent expiration dates.

B.5 Protective gloves, a lab coat, and a mask must be worn during plate setup to prevent contamination.

B.6 Decontaminate the bench work area with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner.

B.7 All kit components of each new lot of GlobalFiler must undergo quality control testing prior to being used for the analysis of casework samples. Do not refreeze kit components after thawing.

The 007 positive control and/or 2800M positive control (Promega) and an amplification negative control will be amplified using the kit components of the new lot of GlobalFiler. The amplified DNA fragments will be separated by electrophoresis on the AB 3130 or 3500xL Genetic Analyzer; sizing and typing will be performed using the allelic ladder from the new kit undergoing quality control testing. The resulting data will be subsequently analyzed using GeneMapper ID-X software. The results obtained from the 007 and/or 2800M samples and allelic ladder must be as expected and good quality as described in the GlobalFiler interpretation guidelines. It may be necessary to amplify multiple concentrations of 007 and/or 2800M in order to obtain optimum quality data. The negative control must exhibit no alleles.

The GeneScan 600 LIZ size standard will pass quality control testing if the appropriate 26 DNA sizing fragments are present in the standard (between 60-460 bp) and these fragments are being utilized appropriately for sizing using GeneMapper ID-X software, e.g. no shoulders or additional peaks are causing off ladder allele calls in the 007 or 2800M positive controls.

The quality control data will be placed into the critical reagent binder.

B.8 Each new lot of TE^{-4} must undergo quality control testing prior to being used to dilute casework samples.

Quality Control Testing: A known sample will be diluted with the TE^{-4} undergoing quality control testing along with an amplification negative control containing TE^{-4}. These samples will be carried through GlobalFiler amplification and electrophoresis. The TE^{-4} will pass quality control testing when a good quality DNA profile with the correct results is obtained for the diluted sample (as described in the GlobalFiler interpretation guidelines) and the amplification negative control is free from contaminants. The quality control data will be placed into the critical reagent binder.

B.9 Amplification setup must be performed in the pre-amplification room. Amplification plate bases should not be brought into the post amplification room.

C. SAFETY

C.1 Protective gloves, a lab coat, and a mask must be worn during plate setup. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn if this procedure is performed outside of a hood.

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

C.3 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS AND CONTROLS

D.1 GlobalFiler

Pre-amplification:

D.1.1 GlobalFiler Master Mix
D.1.2 GlobalFiler Primer Set
D.1.3 DNA Control 007 (0.1 ng/µL)

Post-amplification:

D.1.4 GlobalFiler Allelic Ladder Mix
D.1.5 GeneScan 600 LIZ Size Standard v2.0

D.2 Bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner

D.3 70% Reagent Alcohol (Decontamination)

D.4 TE^{-4} (10mM Tris-HCl- 0.1mM EDTA, 1L)

Add 10mL 1 M Tris-HCl, pH 8.0 and 150µl 0.5 M EDTA to 990mL deionized water. Store at room temperature.

E. EQUIPMENT & SUPPLIES

E.1 Equipment
- E.1.1 Thermal cycler
- E.1.2 Microcentrifuge
- E.1.3 Pipettes
- E.1.4 Vortexer
- E.1.5 96-well plate centrifuge

E.2 Supplies
- E.2.1 Kimwipes
- E.2.2 Sterile aerosol-resistant tips
- E.2.3 Microcentrifuge tubes racks
- E.2.4 AB optical 96-well plates / 0.2 mL tubes
- E.2.5 AB optical strip caps
- E.2.6 96-well plate base
- E.2.7 Disposable gloves
- E.2.8 Mask
- E.2.9 Lab coat
- E.2.10 Eye protection (e.g. safety glasses, face shield)
- E.2.11 Permanent marker

F. PROCEDURES

F.1 Ensure that the standard curve utilized to determine sample concentration meets the requirements in DOC ID 1785 prior to determining the volume of DNA to amplify. When possible target approximately 0.25 -0.75 ng (0.5-1.0 ng for 3500xL platform). Amounts greater than 0.75 ng (1.0 ng for 3500xL) may also be utilized if for example, a mixture is indicated, a sample is degraded, etc. Fill out an amplification sheet. If 50% or less of a sample has been consumed and the quantity of DNA is less than 0.25 ng (0.5 ng for 3500xL), amplification will not be performed; instead, upon careful consideration, additional sample, up to 50%, will be taken. In addition, if a sample has been consumed and the total quantity of DNA that can be input into a reaction is less than 0.010 ng, amplification with GlobalFiler does not have to be performed.

F.2 Dilute samples with TE^{-4} as needed.

F.3 Determine the number of reactions to be amplified. This should include positive and negative control reactions. Add additional reactions to this number to compensate for loss during pipetting.

F.4 Place the required number of 0.2 mL reaction tubes into a rack, and label appropriately. Alternatively, a 96 well plate with labeled wells may be utilized.

F.5 Mix GlobalFiler reagents by vortexing each tube for at least 3 seconds prior to use; these tubes may be centrifuged briefly before opening. Prepare the PCR amplification mix as shown in Table 1 below.

F.6 **Table 1. PCR Amplification Mix for the GlobalFiler System.**

PCR Amplification Mix Component	Volume Per Reaction
TE^{-4}	To a final volume of 25.0 µL
GlobalFiler Master Mix	7.5 µL
GlobalFiler Primer Pair Mix	2.5 µL
Template DNA (0.25-0.75 ng)	Up to 15.0 µL
Total reaction volume	**25.0 µL**

F.7 For each reaction add the appropriate quantity of TE^{-4} to each tube / plate well so that when the sample is added, both components will add up to 15.0 µL.

F.8 Vortex the PCR amplification mix for approximately 3 seconds, then centrifuge briefly. Dispense 10.0 µL of PCR amplification mix into each reaction tube / well.

F.9 Pipet the template DNA for each sample into the respective tube/well containing TE^{-4} and PCR amplification mix.

F.10 For the positive amplification control, add 7.5 µL of 007 (0.1 ng/µL manufacturer's concentration), 7.5 uL of a 1:100 dilution of 2800M (10 ng/uL manufacturer's concentration) or 7.5 uL of 0.1 ng/uL 2800M to the reaction to target 0.75 ng of DNA. It is acceptable to target different amounts of 007 or 2800M DNA for amplification if desired.

F.11 For the negative amplification control, pipet 15.0 µL of TE^{-4} into the appropriate reaction containing PCR amplification mix.

F.12 Close tubes or cover wells with plastic strip caps.

F.13 Place the amplification tubes / plate into a thermal cycler and start the GlobalFiler method. Amplification takes approximately 1.5 hours.

DNA thermal cycling conditions:

Initial incubation:	95 °C for 1 minute
Cycle (29 cycles):	94 °C for 10 seconds (Denature)
	59 °C for 90 seconds (Anneal/Extend)
Final extension:	60 °C for 10 minutes
Final hold:	4 °C

F.14 After the amplification is complete, remove the tubes / plate from the instrument block and store the amplified products in a refrigerator protected from evaporation, e.g. these tubes and plates can be wrapped in parafilm.

***Note**: The amplified products can remain on a thermal cycler overnight or over a weekend at 4°C.*

G. INTERPRETATION GUIDELINES

Not applicable

H. REFERENCES

H.1 GlobalFiler PCR Amplification Kit – PCR Setup (Quick Reference), 2015, Applied Biosystems by ThermoFisher Scientific, Inc.

H.2 GlobalFiler PCR Amplification Kit User Guide, 2014, Applied Biosystems by Life Technologies; Developmental Validation included on pages 54-135.